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(पहला पुनरीक्षण)

Indian Standard

SPICES AND CONDIMENTS — GREEN PEPPER CANNED IN BRINE — SPECIFICATION

(First Revision)

ICS 67.220.10

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

October 2011 **Price Group 6**

Spices and Condiments Sectional Committee, FAD 9

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Spices and Condiments Sectional Committee had been approved by the Food and Agriculture Division Council.

International trade in green pepper canned in brine is on the increase and a need was felt for a specification defining the quality of this product. It is felt that this standard would fulfil the long felt need and ensure a quality product for promoting international trade.

This standard was originally published in 1992. In this standard, the requirements have been updated to align with the ISO Standard on the subject, ISO 11162 : 2001 'Peppercorns (*Piper nigrum* L.) in brine — Specification and test methods'.

Due consideration has also been given to the *Prevention of Food Adulteration Rules*, 1955 and *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977. However, this standard is subject to restrictions imposed under these rules, wherever applicable.

A scheme for labelling environment friendly products known as ECO-Mark has been introduced at the instance of the Ministry of Environment and Forests (MEF), Government of India. The ECO-Mark shall be administered by the Bureau of Indian Standards (BIS) under the *BIS Act*, 1986 as per the Resolution No. 71 dated 20 February 1991 and Resolution No. 425 dated 28 October 1992 published in the Gazette of the Government of India. For a product to be eligible for marking with the ECO-Mark it shall also carry the Standard Mark of BIS for quality besides meeting additional environment friendly (EF) requirements. The environment friendly requirements for green pepper canned in brine are, therefore, also included in this standard.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

SPICES AND CONDIMENTS — GREEN PEPPER CANNED IN BRINE — SPECIFICATION

(First Revision)

1 SCOPE

This standard specifies the requirements, methods of sampling and test of fresh green pepper (*Piper nigrum* L.) canned in brine.

2 REFERENCES

The following standards contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
460 (Part 2) : 1985	Specification for test sieves: Part 2 Perforated plate test sieves (*third revision*)
1070 : 1992	Reagent grade water (*third revision*)
1797 : 1985	Methods of test for spices and condiments (*second revision*)
2860 : 1964	Methods of sampling and test for processed fruits and vegetables
5403 : 1999	Method for yeast and mould count of foodstuffs (*first revision*)
5887 (Part 5) : 1999/ISO 6579 : 1993	Methods for detection of bacteria responsible for food poisoning: Part 5 Isolation, identification and enumeration of Vibrio cholerae and Vibrio parahaemolyticus (*first revision*)
13145 : 1993	Spices and condiments — Methods of sampling (*first revision*)

3 TERMINOLOGY

3.1 Head Space — The distance between the top of the double seam and the level of the surface of the contents.

3.2 Pinheads — Pinheads represent very small, underdeveloped berries of pepper.

4 DESCRIPTION

Canned green pepper consists of fresh green pepper berries of the plant *Piper nigrum* L. belonging to the family *Piperaceae*. The product shall be prepared from fresh green pepper berries at the proper stage of maturity and with similar varietal characteristics.

5 REQUIREMENTS

5.1 Hygienic Requirements

The material shall be prepared and handled under strict hygienic conditions by persons free from contagious and infectious diseases and only in premises maintained in a thoroughly clean and hygienic condition and having adequate and safe water supply and duly approved and licensed by the Public Health Authority. All workers shall use clean, white, washed clothing. Necessary precautions shall be taken to prevent incidental contamination of the product from soiled equipment or from personnel suffering from injuries. All equipment coming in contact with raw materials or products in the course of manufacture shall be kept clean. Ample supply of steam and water, hose, brushes and other equipment necessary for proper cleaning of machinery and equipment shall be available. The equipment may be sterilised by immersion or swabbing with hypochlorite or other suitable chlorine solution. The equipment shall then be washed to remove traces of chlorine.

5.2 Requirements for Covering Brine

The covering brine shall be clear and free from sediment. The only substances that may be added to the covering brine are edible common salt (sodium chloride) with a small quantity of citric or tartaric acid not exceeding 0.6 percent of the packing medium when determined by the method specified in Annex A. The *p*H of brine shall be between 4.0-4.5 when determined using a *p*H-meter. Chloride content of the brine, expressedas sodium chloride shall be between 12-15 percent when determined by the method specified in Annex B.

5.3 Requirements for the Finished Product

The contents of the can on opening shall display the following characteristics.

5.3.1 *Colour*

The units shall possess a good, practically uniform colour, characteristic of green pepper of proper maturity, which means that the colour shall be pale green to green. The units shall be practically free from any discolouration due to improper handling or processing or other causes.

5.3.2 *Texture and Uniformity of Size*

The unit shall possess a tender texture, which means that the units shall be just firm but not hard and shall be characteristic of green pepper which are at the proper stage of maturity. The units shall be uniform in size.

5.3.3 *Taste and Flavour*

The units shall possess taste and flavour characteristic of fresh green pepper. The units shall be completely devoid of any objectionable taste, smell or odour, such as sulphide odour and staleness.

5.3.4 *Extraneous Matter*

For the purposes of this sandard, all materials other than green pepper berries, irrespective of whether they are of vegetable (for example stem or leaves) or mineral (for example sand) origin, shall be considered as extraneous matter.

NOTE — Light berries, pinhead or broken berries are not considered as extraneous matter.

The total percentage of extraneous matter, when determined by the method specified in **4** of IS 1797, shall not exceed 1.0 percent (*m*/*m*).

5.3.5 *Defective Berries*

The maximum limit for defective berries, including discoloured, dark coloured, light and broken berries and pinheads, shall be 4.0 percent (*m*/*m*) when determined on a sample of (drained mass) by physical separation.

5.3.6 *Freedom from Moulds, Insects, Preservatives, Colouring Matter and Flavouring Agents*

Peppercorns in brine shall be free from moulds and insects. They shall also be free from preservatives, colouring matter and flavouring agents unless permitted under the *PFA Rules*, 1955.

5.4 The material shall also comply with the requirements specified in Table 1.

5.5 Pesticide residues in the product shall not exceed the limits as prescribed in the *Prevention of Food Adulteration Act*, 1954 and the Rules made thereunder.

5.6 Additional Requirements for ECO-Mark

5.6.1 *General Requirements*

5.6.1.1 The product shall conform to the requirements of quality prescribed under **5.1** to **5.5**.

5.6.1.2 The manufacturer shall produce the consent clearance as per the provisions of *Water (PCP) Act*, 1974, *Water (PCP) Cess Act*, 1977 and *Air (PCP) Act*, 1981 along with the authorization if required under *Environment (Protection) Act*, 1986 and the Rules made thereunder to the Bureau of Indian Standards while applying for the ECO-Mark and the product shall also be in accordance with the *Prevention of Food Adulteration Act*, 1954 and the Rules made thereunder. Additionally, FPO 1955 (Fruit Product Order) framed under *Essential Commodities Act*, 1955, *Standards of Weights and Measures Act*, 1977 requirements, wherever applicable has to be complied with.

5.6.1.3 The product/packaging may also display in brief the criteria based on which the product has been labelled environment friendly.

5.6.1.4 The material used for product/packing shall be recyclable or bio-degradable.

5.6.1.5 The date of manufacture and date of expiry shall

Table 1 Requirements for Canned Green Pepper

(*Clauses* 5.4, 5.6.2.1 and 8)

Sl No.	Characteristics	Requirements	Method of Test, Ref to	
			Clause of IS 2860	Annex
(1)	(2)	(3)	(4)	(5)
i)	Vacuum in the can in mm, *Min*	125	—	C
ii)	Head space in the can in mm, *Max*	16	—	D
iii)	Drained mass, percent by mass, *Min*	50.0	—	E
iv)	Arsenic, ppm, *Max*	1	**13**	—
v)	Lead, ppm, *Max*	2.5	**14**	—
vi)	Copper, ppm, *Max*	30	**15**	—
vii)	Zinc, ppm, *Max*	19	**16**	—
viii)	Tin, ppm, *Max*	250	**17**	—
ix)	Microbial requirements	To satisfy the requirements of the test	—	F

be declared on the product/package by the manufacturer.

5.6.1.6 The product shall be microbiologically safe when tested as per IS 5403 and IS 5887 (Part 5) and shall be free from bacterial and fungal toxins.

5.6.1.7 The pesticide residues, if any in the product shall not exceed the limit as prescribed in *PFA Act*, 1954 and the Rules made thereunder.

5.6.1.8 The product/package or leaflet accompanying it may display instructions of proper use, storage and transport (including refrigeration temperature compliance) so as to maximize the product performance, safety and minimize wastage.

5.6.2 *Specific Requirements*

5.6.2.1 The product shall not contain any of the heavy metal contaminants in excess of the quantities prescribed in Sl No. (iv) to (viii) of Table 1.

6 PACKING AND MARKING

6.1 Packing

6.1.1 The material shall be packed in tin plate cans. The cans shall be either plain or internally lacquered with food grade lacquer and hermetically sealed. When lacquered externally, the portion under the label except the side seam shall also be lacquered. When lacquered internally, the lacquer shall be sulphur resistant and shall not be destroyed, altered or its components transferred to the material inside the can during the processing or subsequent storage or transport. The exterior of the cans shall be free from dents, rust, perforations and seam distortions. The cans shall not show leaking, panelling or swell. The interior of lacquered cans shall not show any visible black discolouration, rusting or pitting. The interior of the plain cans may show visible dark discolouration. Normal feathering shall not be considered as a defect.

6.1.2 The size of the cans and the net weight of their contents shall generally be as given in Table 2. In case containers other than those specified in Table 2 are used, the size of the containers and the net weight of the contents shall be as agreed to between the purchaser and the vendor.

6.1.3 *Packing in Cases*

The cans shall be packed in cases as agreed to between the purchaser and the vendor.

6.2 Marking

6.2.1 The following particulars shall be legibly and indelibly marked or labelled on each can of green pepper canned in brine:

a) Name, type of the material with the brand name, if any;
b) Name and address of the producer;
c) Net mass of contents;
d) Date of manufacture or code number indicating the date of manufacturer;
e) List of additives, if used;
f) Any other information asked for by purchaser, such as the year of harvest and the date of packing (if they are known); and
g) Any other marking as required under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977 and the *Prevention of Food Adulteration Rules*, 1955.

6.2.2 Each packing case shall also be marked with the following information:

a) Name of the product;
b) Name of the manufacturer;
c) Number of cans and weight of each can;
d) Date of manufacture or code number indicating the date of manufacture; and
e) Date of expiry.

6.3 ECO-Mark

The product may also be marked with the ECO-Mark, the details of which may be obtained from the Bureau of Indian Standards.

Table 2 Size Capacities of Cans

(*Clause* 6.1.2)

Sl No. (1)	Container (Trade-Name) (2)	Trade Size (3)	Nominal Diameter mm (4)	Nominal Height mm (5)	Net Weight of Contents g (6)
i)	No. 1 tall	301 × 409	77.8	115.9	450
ii)	1 lb butter size	401 × 300	103.2	76.2	450
iii)	A-2 size	307 × 408	87.3	114.3	540
iv)	A-2½ size	401 × 411	103.2	119.1	790
v)	A-10	603 × 700	167.2	177.8	2 900

7 SAMPLING

Representative samples of the material shall be drawn and tested for conformity to this standard as prescribed in IS 13145.

8 METHODS OF TEST

The samples of green pepper canned in brine shall be tested for ascertaining conformity of the material to the requirements of this standard in accordance with the relevant clauses given in co1 4 of Table 1.

9 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which effect the results of analysis.

ANNEX A

(*Clause* 5.2)

DETERMINATION OF TOTAL ACIDITY, EXPRESSED AS CITRIC ACID

A-1 PRINCIPLE

The total acidity of brine is determined by titration with sodium hydroxide, using phenolphthalein as indicator.

A-2 REAGENTS

Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

A-2.1 Ethanol, 95 percent to 96 percent (volume fraction).

A-2.2 Phenolphthalein Solution — Dissolve approximately 2 g of powdered phenolphthalein in 1 litre of ethanol (**A-2.1**). Take 10 ml of this solution, using a pipette (**A-3.2**), then dilute to 1 litre with water.

A-2.3 Sodium Hydroxide Solution, 0.1 mol/l.

A-3 APPARATUS

Usual laboratory apparatus and, in particular, the following.

A-3.1 Beaker, graduated at least at 50 ml and 100 ml, class A.

A-3.2 Pipette, class A, to deliver 10 ml.

A-3.3 Burette, of capacity 25 ml.

A-3.4 Magnetic Stirrer

A-3.5 Analytical Balance, capable of weighing to the nearest 0.001 g.

A-4 PROCEDURE

A-4.1 Test Portion

To ensure that the delivery from the burette is between 5 ml and 20 ml, the test portion, weighed to the nearest 0.001 g in the 100 ml beaker (*see* **A-3.1**), shall be in accordance with the following inequality:

$$a = \frac{5}{a} \leq m \leq \frac{10}{a}$$

where

m = mass of the test portion, in g; and

a = supposed total acidity, expressed as a mass fraction of citric acid, in percent.

A-4.2 Determination of Citric Acid Content

Fill the beaker containing the test portion up to the 50 ml mark with phenolphthalein solution (*see* **A-2.2**). Carry out two titrations, with stirring, with the sodium hydroxide solution (*see* **A-2.3**) until the phenolphthalein turns to pink at the endpoint.

A-4.3 Expression of Results

That total acidity, a, expressed as a mass fraction of anhydrous citric acid, in percent, is

$$a = \frac{M \times C_s \times V}{3 \times m}$$

where

M = molar mass of citric acid, that is 192.13;

C_s = is the exact concentration of the sodium hydroxide used in the titration, in moles per litre;

V = volume of sodium hydroxide (titrant) required to reach the endpoint, in litre; and

m = mass of the test portion, in g.

NOTE — Since citric acid is a triacid, the equivalent volume for a given number of moles is three times greater than that for the same number of moles of acetic acid (a monoacid). Because the ratio of molecular masses of citric acid and acetic acid is 192.13/60.04 = 3.2, the acidity expressed as citric acid (a) will give a value which is very close to that expressed as acetic acid (b), the relation can be expressed by the equation:

$$a/b = 1.07$$

ANNEX B

(*Clause* 5.2)

DETERMINATION OF CHLORIDE CONTENT, EXPRESSED AS SODIUM CHLORIDE

B-1 INTRODUCTION

This Annex specifies a method for the determination of the chloride ions present in the brine of peppercorns. The chloride ions are precipitated by titration with silver nitrate. The endpoint is determined potentiometrically using an automatic titration apparatus.

B-2 REAGENTS

Use only reagents of recognized analytical grade.

B-2.1 Water, distilled or demineralized or of equivalent quality, with a conductivity of less than 20 S/cm.

B-2.2 Silver Nitrate Solution, of concentration 0.1 mol/l.

B-3 APPARATUS

Usual laboratory apparatus and, in particular, the following.

B-3.1 Potentiometric Titration Apparatus, with a silver electrode and a glass reference electrode, and suitable titration flasks.

B-3.2 Analytical Balance, capable of weighing to the nearest 0.000 1 g.

B-4 PREPARATION OF TEST SAMPLE

Use the brine collected after draining the peppercorns in brine for the determination of net drained mass (*see* Annex E).

B-5 PROCEDURE

B-5.1 Test Solution

Weigh, to the nearest 0.000 1 g, a test portion of about 5 g in a titration flask. Dilute to about 50 ml with water (*see* **B-2.1**).

B-5.2 Determination

Titrate automatically the chloride ions in the test solution (*see* **B-5.1**) with silver nitrate solution (*see* **B-2.2**), bearing in mind that 1 ml of this solution is equivalent to 5.844 mg of sodium chloride.

B-6 EXPRESSION OF RESULTS

The chloride content, *(w)*, expressed as sodium chloride as a mass fraction in percent, is obtained from the following equation:

$$w = 0.584\,4 \times \frac{V_{eq}}{m}$$

where

V_{eq} = volume of silver nitrate needed to reach the endpoint, in ml; and

m = mass of the test portion, in g.

ANNEX C

[*Table* 1, *Sl No.* (i)]

DETERMINATION OF VACUUM OF THE CANS

C-1 PROCEDURE

The vacuum in the cans shall be determined with an electric recording type machine without opening the can. If such a machine is not available, a vacuum gauge of the piercing type shall be used. Report the vacuum in millimetres of mercury. Necessary corrections for altitude may be made.

ANNEX D

[*Table* 1, *Sl No.* (ii)]

DETERMINATION OF HEAD SPACE

D-1 GENERAL

Either of the two methods, namely, Method I (*see* **D-2**) and Method II (*see* **D-3**) may be used. Method I shall be the referee method in case of dispute.

D-2 METHOD I

D-2.1 Apparatus

D-2.1.1 *Rotary Cutter*

D-2.1.2 *Measuring Stick* — A clean straight flat piece of wood of suitable type not more than 3.2 mm in thickness and 6.4 mm in width.

D-2.2 Procedure

Cut out the lid on the edge of the end-plate partially by the rotary cutter, and lift the cut portion carefully so that the shape of the end-plate is not altered. Introduce the measuring stick in the can and make certain that it goes straight in and rests flat against the bottom of the can and against the body plate. Rest for a short time and lift the stick straight. Find out the length of the stick which is wet, the space occupied by the contents of the can. Empty out the contents of the can carefully and fill it completely with water. Again introduce a similar measuring stick and find out the length of the stick which is wet. The difference between the readings gives the gross head space. Net head space is equal to gross head space less 5 mm. Take four recordings. The average of the four net head space recordings taken at different points shall be the head space of the can.

D-3 METHOD II

D-3.1 Apparatus

D-3.1.1 *Rotary Cutter*

D-3.1.2 *Ruler* — With millimetre divisions.

D-3.2 Procedure

Cut out the lid on the edge of the end-plate with the rotary cutter, and lift the cut portion carefully so that the shape of the end-plate is not altered. Take the measurement from the top of the double seam to the surface of the liquid using a ruler. The average of four measurements taken at different points shall be the head space of the can.

ANNEX E

[*Table* 1, *Sl No.* (iii); and *Clause* B-4]

DETERMINATION OF DRAINED WEIGHT

E-1 APPARATUS

E-1.1 1.70-mm IS Sieve (Aperture 1.70 mm) — Alternatively, BS Sieve 10 or ASTM Sieve 12 or Tyler Sieve 10 may be used as they are equivalent to 1.70 mm IS Sieve [*see* IS 460 (Part 2)].

E-2 PROCEDURE

E-2.1 Carefully weigh the clean and dry sieve and empty the contents of the can into the sieve in such a manner as to distribute the product evenly. Without disturbing the product, incline the sieve so as to facilitate drainage, Allow to drain for two min, and weigh the sieve again along with the product. The drained weight shall be the weight of the product and sieve less the weight of the sieve.

E-2.2 A sieve of size 20.3 cm × 20.3 cm shall be used for A-2½ cans or smaller. In case of cans of size larger than A-2½, a sieve of size 30.5 cm × 30.5 cm shall be used.

ANNEX F

[*Table* 1, *Sl No.* (ix)]

TEST FOR MICROBIOLOGICAL REQUIREMENT

F-1 GENERAL

The test consists of the incubation of the cans at 37°C and 55°C for not less than 14 days and their examination at the end of this period for evidence of microbiological activity. To satisfy the requirements of this test, the cans shall not show evidence of microbiological activity of any type. The incubated cans shall be considered to have undergone microbiological spoilage, if any can,

a) shows a positive pressure;
b) bulges when being incubated or remains bulged after incubation;
c) leaks; and
d) whether having a positive pressure or not, shows evidence of bacterial proliferation as judged by,
    1) a significant change in *p*H value;
    2) disintegration or digestion of the flesh as compared with the unincubated samples;
    3) microscopic examination of direct smears; and
    4) culture methods for testing bacterial multiplication and presence of pathogenic bacteria.

F-2 APPARATUS

F-2.1 Glassware — All the glass apparatus used in the microbiological examination shall be sterile. Sterilization shall be performed for at least 2 h at 170ºC in dry heat.

F-2.2 Vacuum-Pressure Recorder — Preferably with an electric recording type of instrument. Alternatively, a vacuum gauge of the piercing type may be used.

F-2.3 Accessories — Can-opener of the puncturing type, cork borers, sampling tubes, spoons, and cottonwool — all sterile.

F-3 REAGENTS

F-3.1 Petroleum Ether

F-3.2 Mercuric Chloride Solution — 1.0 percent (*m*/*v*).

F-3.3 Carbolic Acid Solution — 1.5 percent (*m*/*v*).

F-3.4 Ethyl Alcohol Solution — 95 percent, 97 per cent and 98 percent (*m*/*v*).

F-3.5 Chloroform

F-3.6 Formaldehyde — 40 percent (*m*/*v*).

F-3.7 Sterile Normal Saline or Ringers Solution

F-3.7.1 *Normal Saline* — 0.9 percent (*m/v*) solution of sodium chloride (analytical reagent grade).

F-3.7.2 *Ringers Solution* — It has the following composition:

Sodium chloride	:	0.2 g
Potassium chloride	:	0.42 g
Calcium chloride	:	0.48 g
Sodium carbonate	:	0.2 g
Distilled water	:	1 000 ml (glass distilled)

The above solution is diluted to quarter strength when actually in use.

F-3.8 Sterile Vaseline — Alternatively, sterile liquid paraffin may be used.

F-4 MEDIA

F-4.1 Dextrose-Tryptone Broth or Dextrose-Nutrient Broth

F-4.1.1 *Dextrose-Tryptone Broth*

Mix 10 g of tryptone, 5 g of dextrose, 0.04 g of bromo-cresol-purple and one litre of water. Steam the mixture until dissolved. Adjust the *p*H to 6.8 to 7.0. Filter and tube in 10-ml quantities and autoclave at 121°C for 30 min.

F-4.1.2 *Dextrose-Nutrient Broth*

Mix 3 g of beef extract, 5 g of peptone, 20 g of dextrose and one litre of water. Warm to dissolve, tube in 10-ml quantities and autoclave at 121°C for 30 min. Alternatively, dissolve 5 g of peptone, 5 g of glucose and 5 g of dipotassium hydrogen phosphate (K_2HPO_4) in water, filter, cool and make up the volume to one litre. Tube in 10-ml quantities and sterilize by steaming for 30 min for 3 successive days, each steaming followed by storage at room temperature.

F-4.2 Dextrose-Tryptone Agar

To the dextrose-tryptone broth prepared as in **D-4.1.1** and 20 g of powdered agar just before sterilization and steam to dissolve. Tube in quantities of 20 to 25 ml and autoclave at 121°C for 30 min.

F-4.3 Sulphite Agar

Mix 1 g of tryptone, one gram of sodium sulphite, 20 g of agar powder, 0.1 g of ferric citrate and one litre of water. Warm to dissolve and tube in 10-ml quantities. Autoclave at 121°C for 30 min. Alternatively, omit adding ferric citrate, adjust the *p*H to 7.0 and add a bright sand-papered iron nail into each tube and sterilize.

F-4.4 Liver Broth or Sodium Thioglycollate

F-4.4.1 *Liver Broth*

Boil 500 g of well-minced degutted beef liver in 100 ml of distilled water for one hour. Adjust the *p*H of the mixture to 7.0 and boil further for 10 min. Cool and separate all fat by straining through several layers of muslin and make up the volume to one litre with water. Add 10 g of peptone, one gram of dipotassium hydrogen phosphate and again adjust the *p*H to 7.0. Tube in 10-ml quantities, add to each tube 2 g of liver particles and autoclave at 121°C for 30 min. Before use, boil the medium for 10 to 15 min to remove dissolved air. Cool the tube before inoculation. The medium may further be improved by sealing with paraffin wax or liquid paraffin.

F-4.4.2 *Sodium Thioglycollate Medium*

To 100 ml of glucose nutrient broth, add one gram of powdered agar and one g of sodium thioglycollate. Autoclave in bulk at 115°C for 20 min. Tube in 10-ml quantities and autoclave at 121°C for 30 min. This medium acts as an anaerobic medium and requires no further preparation or special conditions for use. It can straightaway be used as an anaerobic medium in an ordinary incubator. Alternatively, for a sugar-free base medium useful for fermentation reactions, the following formula may be employed:

Proteose peptone	:	20 g
Sodium chloride	:	5.0 g
Sodium thioglycollate	:	1.0 g
Agar	:	1.0 g
Distilled water	:	1 000 ml

Sugars as required are added up to 1.0 percent to the base just before tubing and sterilization.

F-4.5 Endos Agar or Eosin Methylene Blue Agar

F-4.5.1 *Endos Agar* (*Levines Formula*)

Mix 10 g of peptone, 2.5 g of dipotassium hydrogen phosphate, 15.3 g of agar and one litre of water. Boil the mixture until dissolved. Make up the loss due to evaporation with water. Sterilize at 121°C for 20 min. Just before use, for every 100 ml of solution, add the following solution:

Lactose solution (20 percent, *m*/*v*)	:	5 ml
Basic fuchsine (10 percent, *m*/*v*), alcoholic	:	0.5 ml
Sodium sulphite solution (10 percent, *m*/*v*), freshly prepared	:	2.5 ml

Sterilize for 20 min at 121°C. The basic fuchsine solution should be freshly prepared and added just before sterilization.

F-4.5.2 *Eosin Methylene Blue Agar* (*Holt, Horris and Teague*)

This medium is used for differentiating *Escherichia coli* from *Eberthella typhosa* and has the following composition:

Peptone	:	10 g
Agar	:	15 g
Lactose	:	5 g
Sucrose	:	2 g
Dipotassium hydrogen phosphate	:	2 g
Eosin Y	:	0.2 g (added as 20 ml of 2 percent aqueous solution)
Methylene blue	:	0.05 g (added as 20 ml of 0.5 percent aqueous solution)
Distilled water	:	1 000 ml

If necessary, the *p*H may be adjusted to 6.5 to 7.0.

F-4.6 Brilliant Green Lactose Saccharose Phenol Red Agar or Brilliant Green Lactose Bile

F-4.6.1 *Brilliant Green Lactose Saccharose Phenol Red Agar*

Dissolve 10 g of peptone, 5 g of meat extract, 5 g of sodium chloride and 20 g of agar in 1 000 ml of water by boiling for 30 min. Filter through flannel. Adjust the *p*H to 7.2. Sterilize for 30 min at 110°C. Take 1 000 ml of melted agar medium, add aseptically a solution consisting of 10 g of lactose and 10 g of saccharose in 80 ml of 0.005 N sodium hydroxide solution containing one percent of phenol red. This solution should be sterilized by boiling at 100°C for 10 min. Finally, add 2 ml of brilliant green solution (0.5 percent, *m*/*v*). No resterilization is required.

F-4.6.2 *Brilliant Green Lactose Bile* (*Muer and Harris*)

The medium has the following composition:

Dried oxgall	:	50 g
Peptone	:	10 g
Lactose	:	10 g
Brilliant green	:	0.1 g
Distilled water	:	1 000 ml

The distilled water is heated on a water-bath, the oxgall is added with the peptone and dissolved. Boiling is continued for one hour. The lactose is then added. The solution is filtered and 10 ml of one percent solution of brilliant green is added. The medium is sterilized in tube for 15 min.

F-4.7 MacConkey Broth (Single Strength)

The medium has the following composition:

Sodium taurocholate	:	5 g
Lactose	:	10 g
Peptone	:	20 g
Sodium chloride	:	5 g
Distilled water	:	1 000 ml

Steam the mixture for about 2 h and keep in a refrigerator overnight. Filter in the morning while cold. Adjust *p*H to 7.4 using phenol red as indicator. Add 10 ml of neutral red indicator solution (one percent, *m*/*v*). Distribute the medium into culture tubes in 5-ml portions. The culture tubes should have Durham fermentation tubes in them. Sterilize the medium for 3 days at 10.346 N/m^2 pressure. When ready, the medium should have a light claret red colour and should be free from magenta or yellow tints.

F-4.8 S.S. Agar Medium for *Salmonella* and *Shigella*

The growth of coliform organisms is considerably inhibited on this medium. If growth occurs at all, the colonies are red or pink centred while *Salmonella* and *Shigella* colonies are colourless. The medium has the following composition:

Bile salt agar base (prepared as in **D-4.8.1**)	:	1 000 ml
Lactose	:	10 g
Sodium citrate	:	8.5 g
Sodium thiosulphate	:	8.5 g
Ferric citrate	:	1.0 g
Neutral red (on percent, aqueous)	:	2.5 ml
Brilliant green (0.1 percent, aqueous)	:	0.33 ml

Add the dry ingredients to the hot, melted agar base and mix thoroughly to dissolve. Adjust the *p*H to 7.0. Add the neutral red and brilliant green solutions. Mix well. Do not autoclave. Pour 15 to 20-ml amounts into sterile petridishes and allow the plates to stand with the top partially lifted for two h or so until the surface is quite dry.

F-4.8.1 *Bile Salt Agar Base*

It has the following composition:

Beef extract	:	5 g
Proteose peptone	:	5 g
Bile salts	:	8.5 g
Agar	:	17 g
Distilled water	:	1 000 ml

Dissolve the dry ingredients in water by heating on water-bath or in autoclave. Restore the original volume by adding water. Distribute into flasks in 100-ml portions and autoclave at 121°C for 30 min.

F-4.9 Tetrathionate Broth

This is useful for the primary isolation of *Salmonella* (including the typhoid bacillus) and for inhibiting the growth of coliform organisms. It is prepared as follows.

F-4.9.1 *Tetrathionate Broth Base*

Proteose peptone	:	5 g
Bile salts	:	1 g
Calcium carbonate	:	10 g
Sodium thiosulphate	:	30 g
Brilliant green (0.1 percent, aqueous)	:	11 ml
Distilled water	:	1 000 ml

Dissolve peptone and bile salts in water. Distribute in flasks in 500-ml quantities. Add 5 g of calcium carbonate to each flask. Autoclave at 121°C for 10 min. Add 15 g of sodium thiosulphate and 5.5 ml of brilliant green solution to each flask and mix thoroughly. Distribute aseptically into tubes in 10-ml portions. Keep suspension of calcium carbonate uniform by agitation.

F-4.9.2 *Iodine Solution*

Iodine crystals	:	30 g
Potassium iodide	:	25 g
Distilled water	:	100 ml

Dissolve the ingredients in water.

F-4.9.3 Just before inoculating add 0.2 ml of iodine solution to each of the tetrathionate broth base tubes. Add 1 to 3 g of the specimen to one tube of the medium. Mix thoroughly by swirling and incubate for 24 h. After the full period of incubation, streak on S.S. agar plates and the MacConkey broth.

F-5 PROCEDURE

F-5.1 Preparation of the Can

F-5.1.1 All the identification marks, brand marks, batch number, date and other information available on the label or embossed on the tin shall be noted. The label shall be removed and all physical defects, such as rust, pin-holding, dents, signs or defective seaming of end plates and of defective side seams, shall be recorded and points, if any, for examination after the can is opened shall be noted.

F-5.1.2 The can shall be thoroughly scrubbed with soap and water. If necessary, apply petroleum ether and clean. After washing with soap, immerse in a tall container filled with mercuric chloride solution or carbolic acid solution. The immersion shall be for at least 1 h.

F-5.1.3 After removing from the antiseptic bath, clean the exterior of the can with sterile cotton wool. When dry, scrub well with sterile cotton wool dipped in ethyl alcohol (97 percent). Cover the top of the can with sterile petri dish.

F-5.2 Recording of the Vacuum or Pressure

F-5.2.1 This recording is done preferably with an electric recording type of instrument without opening the can. If such an instrument is not available, the recording may be done with a vacuum-pressure gauge of the piercing type.

F-5.2.2 *Recording of Vacuum or Pressure with a Vacuum-Pressure Gauge*

F-5.2.2.1 The site of the opening to the made shall be thoroughly sterilized by any of the following methods:

a) Pour ethyl alcohol (95 percent), freely on the top-end plate and set fire. Flame the petri dish freely and close the end plate with the dish immediately after the alcohol has burnt down;
b) Hold the can in the hand and heat the end plate freely in a Bunsen flame waving the end plate against the flame with a circular motion so as to protect the contents against scorching. Cover with a sterile petri dish as before; and
c) In case the can is bulged, it is not safe to directly flame it. It can be sterilized by swabbing freely with a mixture of ethyl alcohol (98 percent) and chloroform or formaldehyde after washing with soap and water.

F-5.2.2.2 After flaming, allow the can to remain under the sterile petri dish until quite cool (30 min would do) and then pierce the point of opening by means of a sterile vacuum pressure gauge of the piercing type under aseptic conditions. Record the vacuum or positive pressure as the case may be. Cover the opening immediately after removing the vacuum-pressure gauge.

F-5.3 Preparation of the Sample for Bacteriological Tests

Enlarge under aseptic conditions the puncture with a thoroughly sterilized can-opener to give an opening of 2.5 cm diameter. If a cutting type of instrument is used, cut a triangle with a base of 2.5 cm and sides 5 cm long. Cover the enlarged opening at once with sterile petri dish.

F-5.4 Removal of the Inoculum

F-5.4.1 *Removal of Liquid lnoculum*

The inoculum is especially removed by means of a sterile pipette graduated in millimetres. It is essential that the rear end of the pipette be plugged for a length not less than 1.5 cm with sterile cotton wool. Deliver 2 ml of the inoculum to each of the broth tubes and 1 ml to each of the melted agar tubes. Mix well to ensure uniform distribution and pour into the petri dishes, swirling to as to obtain uniform spread. Alternatively, pour 2 ml of the inoculum into the petri dish and then add the melted agar medium and mix by swirling.

F-5.4.2 *Removal of Solid Inoculum*

For removing the samples of solid material, use sterile cork-borers or sample tubes or spoons. If spoons are used, the contents of the can should be thoroughly mixed before taking the sample. Remove about 15 g of the sample and transfer it to a sterile flask containing 50 ml of sterile water or sterile normal saline or sterile Ringers solution. The flask should contain at least a dozen sterile glass beads to facilitate mixing.

Mix by swirling for at least 5 min. Take out 2-ml portions of the inoculum and add them to both the broths and the agars.

F-5.5 Preparation of the Anaerobic Culture

The dextrose-tryptone broth and the liver-broth media are rendered anaerobic by sealing the access to air by placing a layer of sterile vaseline, liquid paraffin, liquid agar or paraffin wax on the top of the medium. This layer should be not less than 1.5 cm in thickness.

Just before using the culture tubes exhaust the media by packing the tubes for at least 10 min in a water-bath and maintain the water-bath at 70°C until the media are used.

Inoculate the medium below the paraffin-agar layer with as little disturbance as possible and cool the medium after inoculation in a cold water-bath so that the top seal is set.

F-5.6 Incubation

F-5.6.1 Incubate the inoculated tubes as follows:

a) *Dextrose-tryptone broth*, two tubes each, aerobically and anaerobically at 37°C for 3 to 5 days and similarly at 55°C for 3 to 5 days;
b) *Sulphite agar*, one tube each at 37°C and 55°C for 5 days;
c) *Liver broth or sodium thioglycollate*, two tubes anaerobically at 37°C and two tubes at 55°C for 5 days;
d) *Endos agar*, at 37°C for 48 h;
e) *Brilliant green lactose saccharose phenol red agar*, at 37°C for 48 h;
f) *MacConkey broth*, is inoculated with at least 5 ml of the inoculum and incubated at room temperature for 48 h; and
g) *S.S. agar*, is incubated at 37°C for 48 h.

F-5.6.2 In the case of a blown can, run a separate series of culture tubes parallel to the tubes from the sound can. The incubation period is the same as given in **F-5.6.1**.

F-5.7 Examination of the Culture After Incubation

F-5.7.1 In case there is growth in the nutrient broth tubes, streak them on agar plates and incubate. Examine the contents of each culture tube for the organisms of the following types:

a) Spoilage types,
b) Potential spoilage types, and
c) Pathogenic types.

F-5.7.2 In case there is a growth of cocci, establish their nature by streaking them on blood agar and study ability for haemolysis and nature by: (a) coagulase tests, and (b) microscopic examination.

F-5.8 Examination of the Cans After the Removal of Inoculums

F-5.8.1 After the removal of the inoculum from the cans, carry out the following tests:

a) Prepare a direct smear of the contents. Stain by Gram's Method or methyl violet and examine the preparations microscopically;
b) Determine the *p*H value; and
c) Examine the contents for deterioration, discolouration, disintegration and other signs of spoilage.

F-5.8.2 Correlate the above findings with the incidence and nature of microflora and conclude about the cause of the spoilage, if any.

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